Supplemental Figure 1: Hydrocephalus in *lnks/lnks* mutant pups

Saggital sections of 2-3 week old pups from wildtype (a,b) or *lnks/lnks* mutants (c,d). *lnks/lnks* pups display hydrocephalus (asterisks) and die shortly after weaning.

Supplemental Figure 2: Morpholino injections affect splicing of the *ccdc40* transcript

PCR analysis indicates that the ccdc40 e12i12 morpholino (MO1; a) and the i10e11 morpholino (MO2; b) affect splicing of the zebrafish *ccdc40* transcript at different developmental stages. The predicted product in uninjected embryos is indicated with a white asterisk. The multiple bands seen in uninjected controls could indicate alternative splicing of the transcript, but this has not been formally investigated. Given that both morpholinos affect splicing of the *ccdc40* transcript, produce similar defects to each other, phenocopy *lok* mutants, and are rescued by *ccdc40* mRNA coinjection, the defects produced by the morpholinos are most likely specific.

Supplemental Figure 3: Genomic organization of *CCDC40* and location of mutations

(a) Distribution of identified *CCDC40* mutations. The CCDC40 transcript is 4287bp encoding an 1142aa protein Exons are numbered and indicated by black boxes. The

open box represents untranslated region. ATG: start codon; TAG: stop codon. Intron sizes are not drawn to scale. The positions of all identified *CCDC40* mutations are indicated by vertical lines. (b-d) Segregation analysis of family OP-43. (b) The father carries a heterozygous loss-of-function mutation in exon 9, which he transmitted to patient (OP-43 II1; d). SNP analysis within the 5'UTR and intron 1 show that the patient (OP-43 II1; d) solely inherited the nucleotides A (rs3752042; rs3764438) from the father indicating presence of a heterozygous deletion affecting at least the 5'UTR and exon 1 in the patient (OP-43 II1) and the mother (OP-43 I2; c). This segregation pattern is also described as non-parental (maternal) contribution of these SNPs (rs3752042; rs3764438) for the affected child. The deletion does not affect intron 4 as the polymorphism in intron 4 is homozygous for the nucleotides C (rs7212525) for the father (OP-43 I1), homozygous for the nucleotide T for the mother (OP-43 I2) and heterozygous for both nucleotides for the patient (OP-43 II1).

Supplemental Figure 4: Sequence analyses for *CCDC40*

(a) Sequence chromatographs depicting the homozygous deletion for OP-120. (b) Sequence chromatographs demonstrates the homozygous loss-of-function mutation (c.3129delC) predicting a premature stop of translation (p.F1044Sfs35X) for the affected person OP-76 II1. The mother (OP-76 I2) is a heterozygous carrier for this mutation. Segregation of the mutant alleles is consistent with the assumption that the other mutated allele is inherited through the father which is consistent with autosomal recessive inheritance. (c) Sequence chromatographs showing the homozygous loss-of-function mutation (c.C1315T) predicting a premature stop of translation (p.Q439X). The parents are heterozygous carriers for the mutation which is consistent with autosomal recessive inheritance and homozygosity by descent. (d) Sequence chromatographs showing the deletion for OP-712. (e) Parents of patient OP-57 II are heterozygous carriers for the loss-of-function mutation c.C1971T which is consistent with autosomal recessive inheritance. (f-g) Sequence chromatographs show the mutation located within exon 3 and exon 12 of *CCDC40*. The patient OP-82 II1 carries two heterozygous loss-of-function mutations (c.248delC and c.C1810T) predicting a premature stop of translation (p.A83Vfs82X and p.Q604X). The father is a heterozygous carrier for the

mutation in exon 12 (g). The mother is a heterozygous carrier for the mutation in exon 3 (f). This is consistent with autosomal recessive inheritance.

Supplemental Figure 5: Segregational analyses in family OP-43

(a) Sequence chromatographs show that OP-43 II1 inherited a heterozygous loss-of-function mutation (c.C1366T) predicting a premature stop (p.R449X) from the father. (b) The 5'UTR sequence chromatograph of patient (OP-43 II1) shows solely the A-allele at position c.-49, that is inherited by the father (OP-43 I1). The mother (OP-43 I2) carries solely the C-allele at this position indicative for parental non-contribution. (c) SNP analysis within intron 1 is also consistent with maternal non-contribution indicating presence of a large heterozygous deletion present in the patient (OP-43 II1) and the mother (OP-43 I2). (d) Sequence chromatographs show that the sequences for the polymorphisms in intron 4 are homozygous for the nucleotide C for OP-43 I1, the mother (OP-43 I2) is homozygous for the nucleotide T, whereas the patient is heterozygous for both nucleotides, demonstrating that the deletion most likely does not affect intron 4. Polymorphisms are marked by black arrows.

Supplemental Figure 6: Beat pattern of *CCDC40* mutant respiratory cilia

Comparison between normal flagellar beat pattern in Chlamydomonas (a) and the beating pattern of flagella with missing PF2 (orthologue of human GAS11) in the axoneme (b). The amplitude in the mutant is markedly reduced. In comparison, the ciliary beat of respiratory cilia in humans with *CCDC40* mutations is severely altered (d); characterized by a reduced amplitude and stiff and rigid cilia. For comparison normal ciliary beat is displayed (c). (Black = effective stroke, Grey = recovery stroke). Illustrations in (a) and (b) are adapted and modified from Kamiya et al [18].

Supplemental Figure 7: Cilia length

(A) Cilia length of respiratory cells from five different healthy controls (number of measurements for controls; n = 33) and seven PCD patients harbouring *CCDC40* mutations were determined. Cilia length in both groups is similar. The length of cilia was

evaluated with the “LSM Image Browser” software. Bars display standard deviation. Number of cilia measurements for patients: OP-799 [n = 15]; OP-712 [n = 15]; OP-741 [n= 18]; OP-659 [n = 18]; OP-57II1 [n = 18]; OP-43II1 [n = 18] and F-727II1 [n = 31]. (B,C) Examples for the measurements of respiratory cilia lengths of a healthy control (B) and a patient OP-799 (C).

Supplemental Figure 8: Ccdc40 WB

Demonstration of specificity of the polyclonal rabbit antibodies targeting Ccdc40.

Western blot analyses demonstrate that anti-Ccdc40 detects a single band of the expected size (~136kDa) in mouse respiratory epithelial cell lysates (lane 1). This band is missing in the lysate from a *links* mutant mouse (lane 2) demonstrating specificity of the antibody. Right panel shows silver staining.

Supplemental Table 1: *CCDC40* Mutations in primary ciliary dyskinesia.

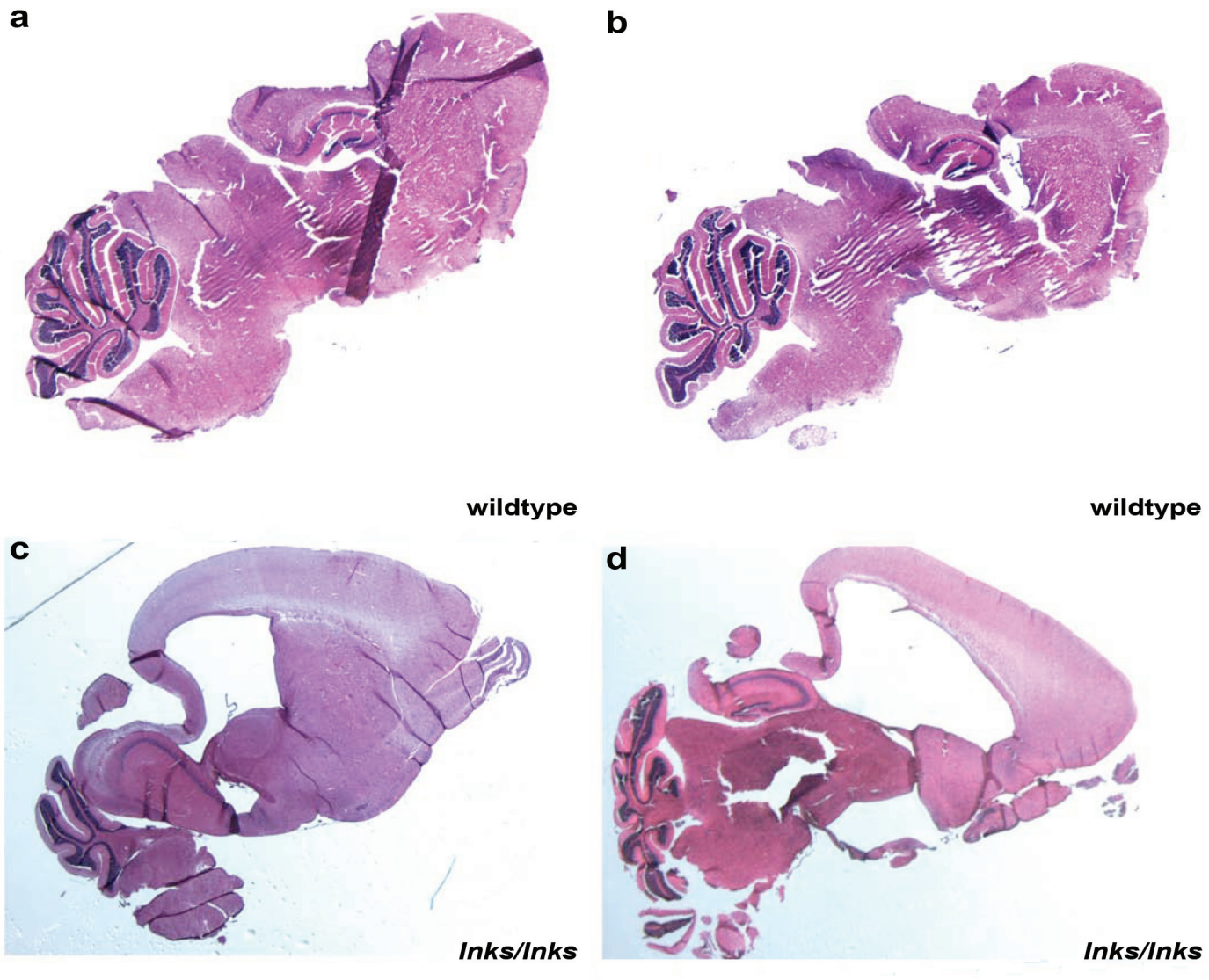
(Expanded Table 1 containing additional data).

Supplementary Videos: Motility of cilia in respiratory cells from a healthy control and patients carrying mutations in *CCDC40*.

The coiled-coil domain containing protein CCDC40 is essential for motile cilia function and left-right axis formation

Anita Becker-Heck#, Irene Zohn#, Noriko Okabe#, Andrew Pollock#, Kari Baker Lenhart, Jessica Sullivan-Brown, Jason McSheene, Niki T. Loges, Heike Olbrich, Karsten Haeffner, Manfred Fliegauf, Judith Horvath, Richard Reinhardt, Kim G. Nielsen, June K Marthin, Gyorgy Baktai, Kathryn V. Anderson, Robert Geisler, Lee Niswander*, Heymut Omran*, Rebecca D. Burdine*

# These authors contributed equally
* corresponding authors who jointly supervised this work



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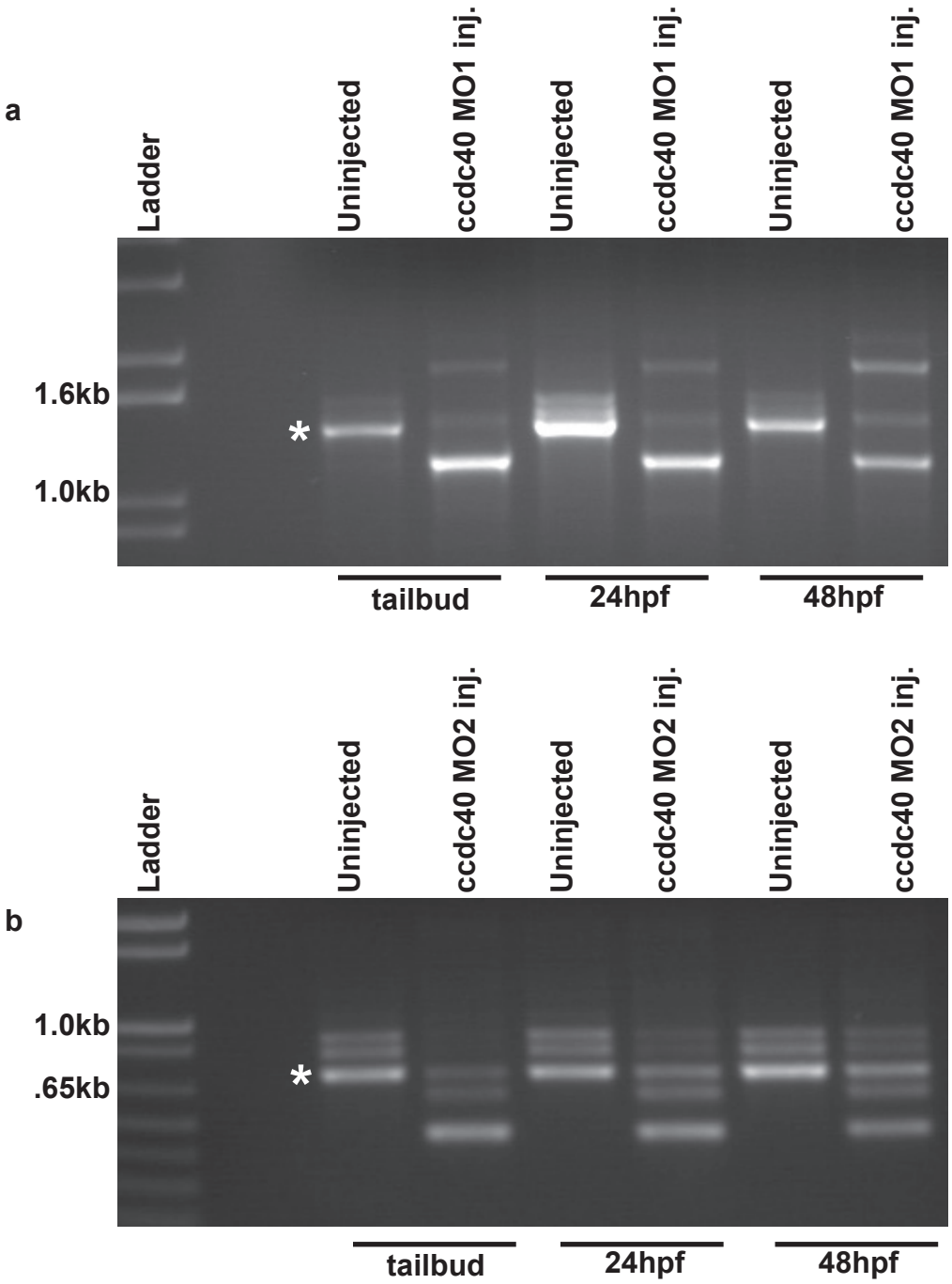

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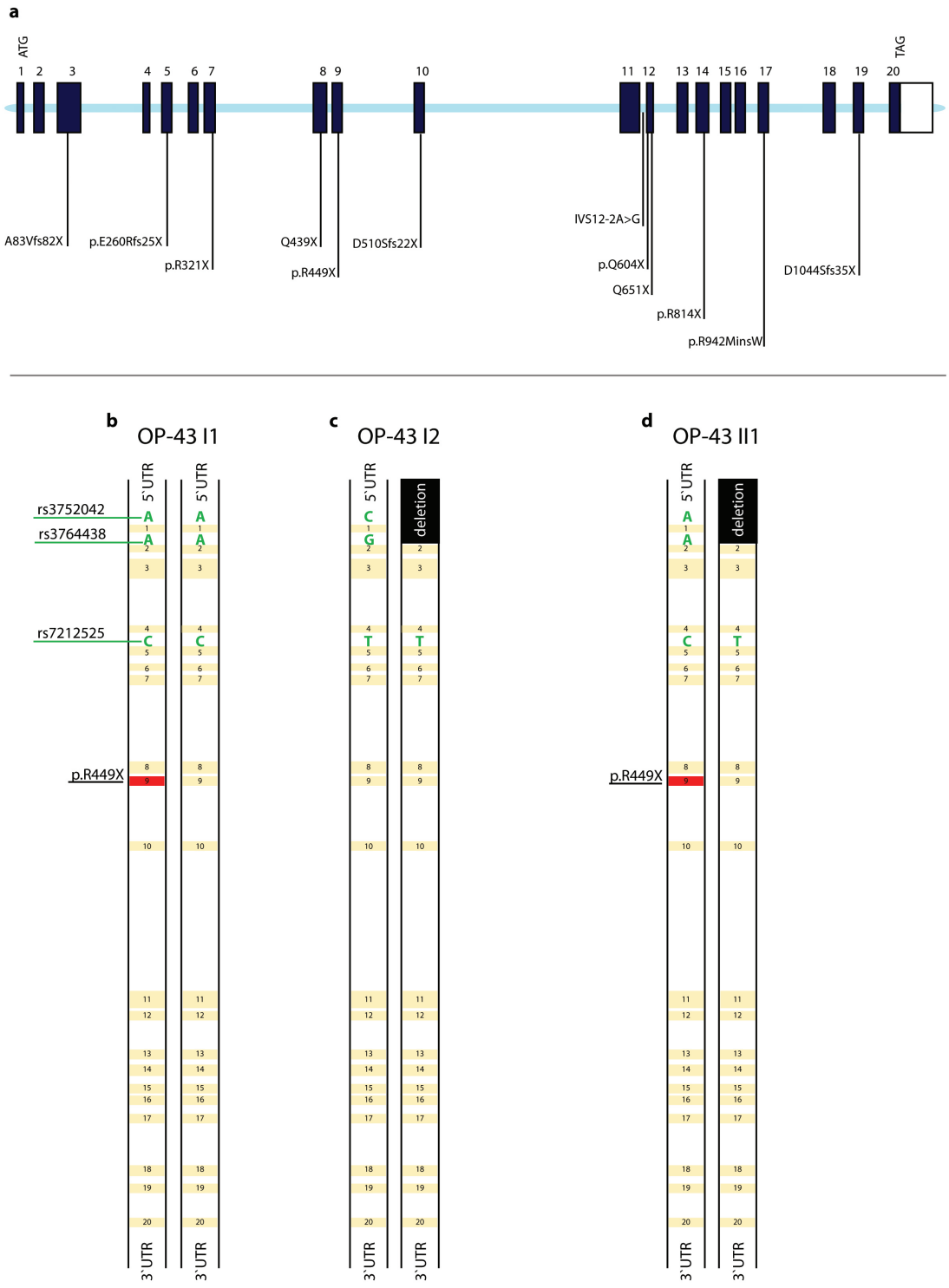

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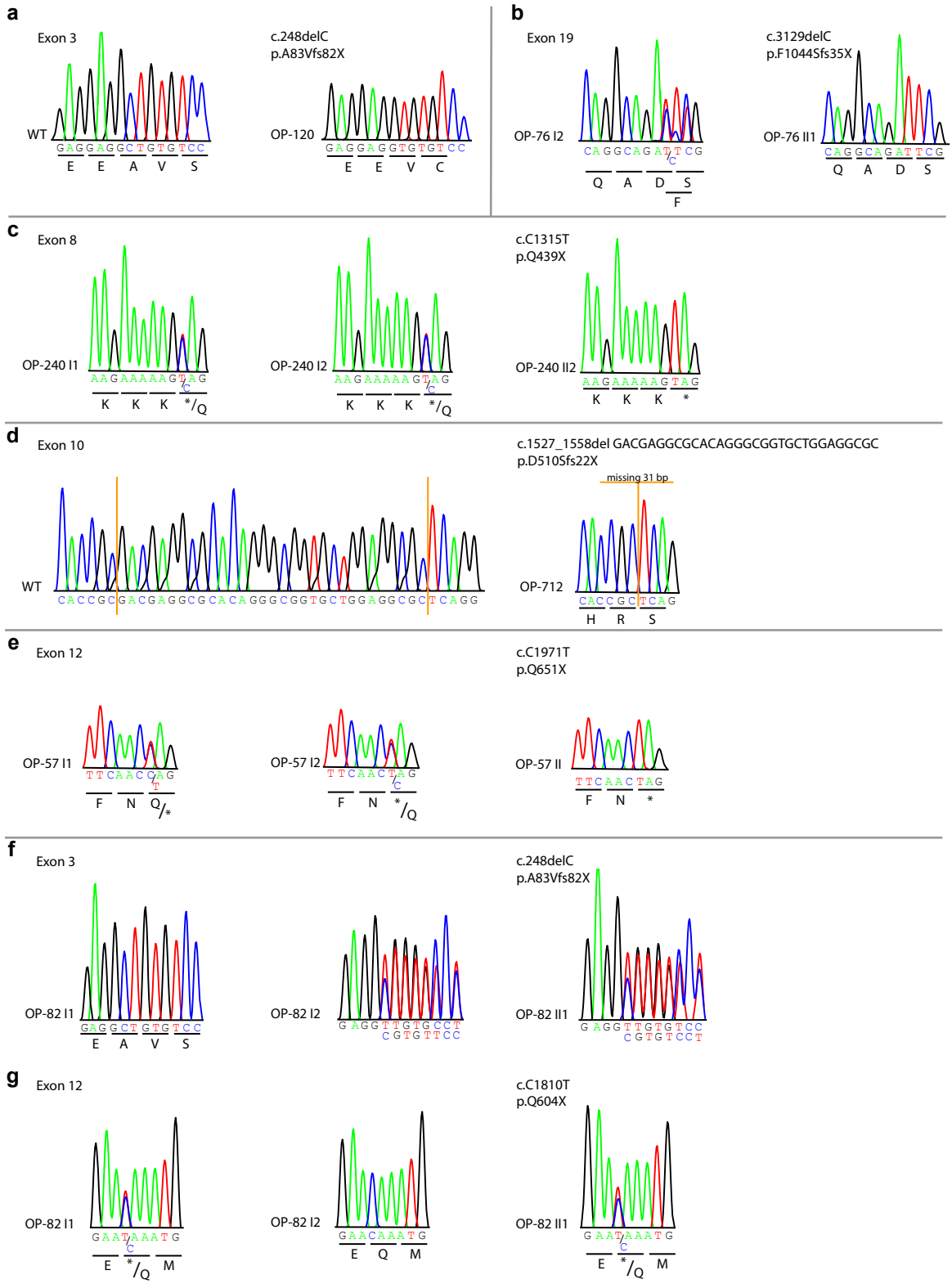

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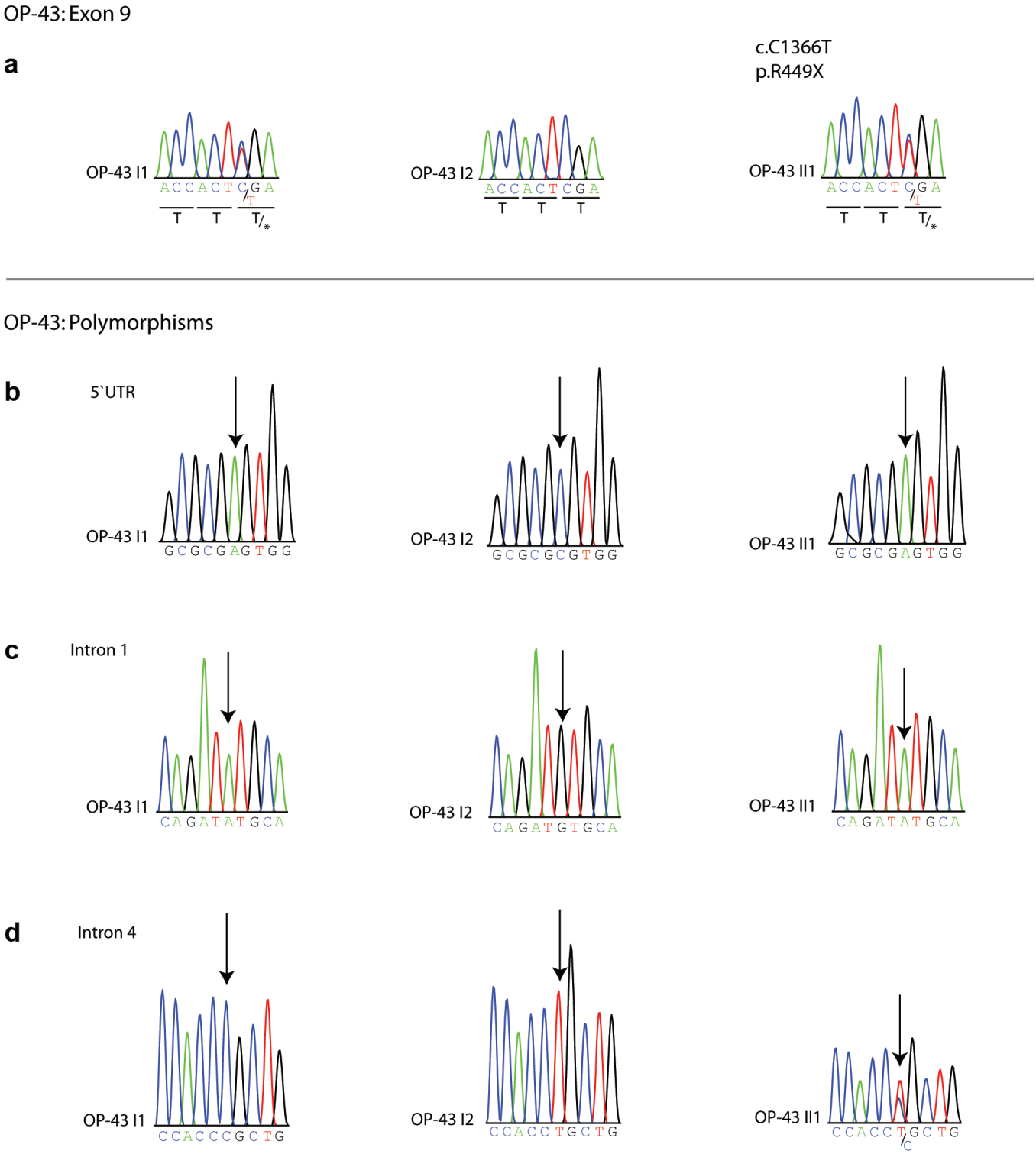

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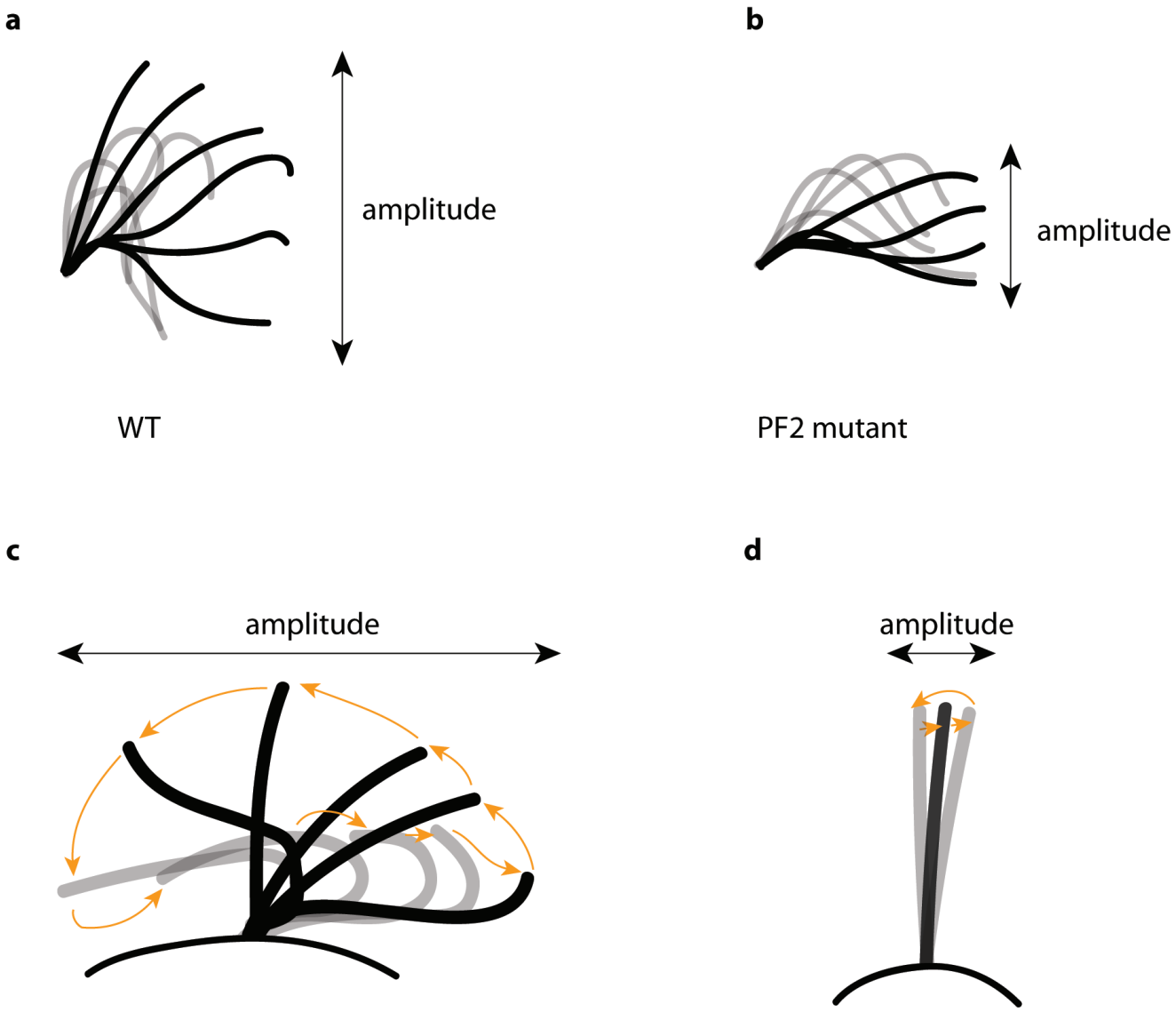

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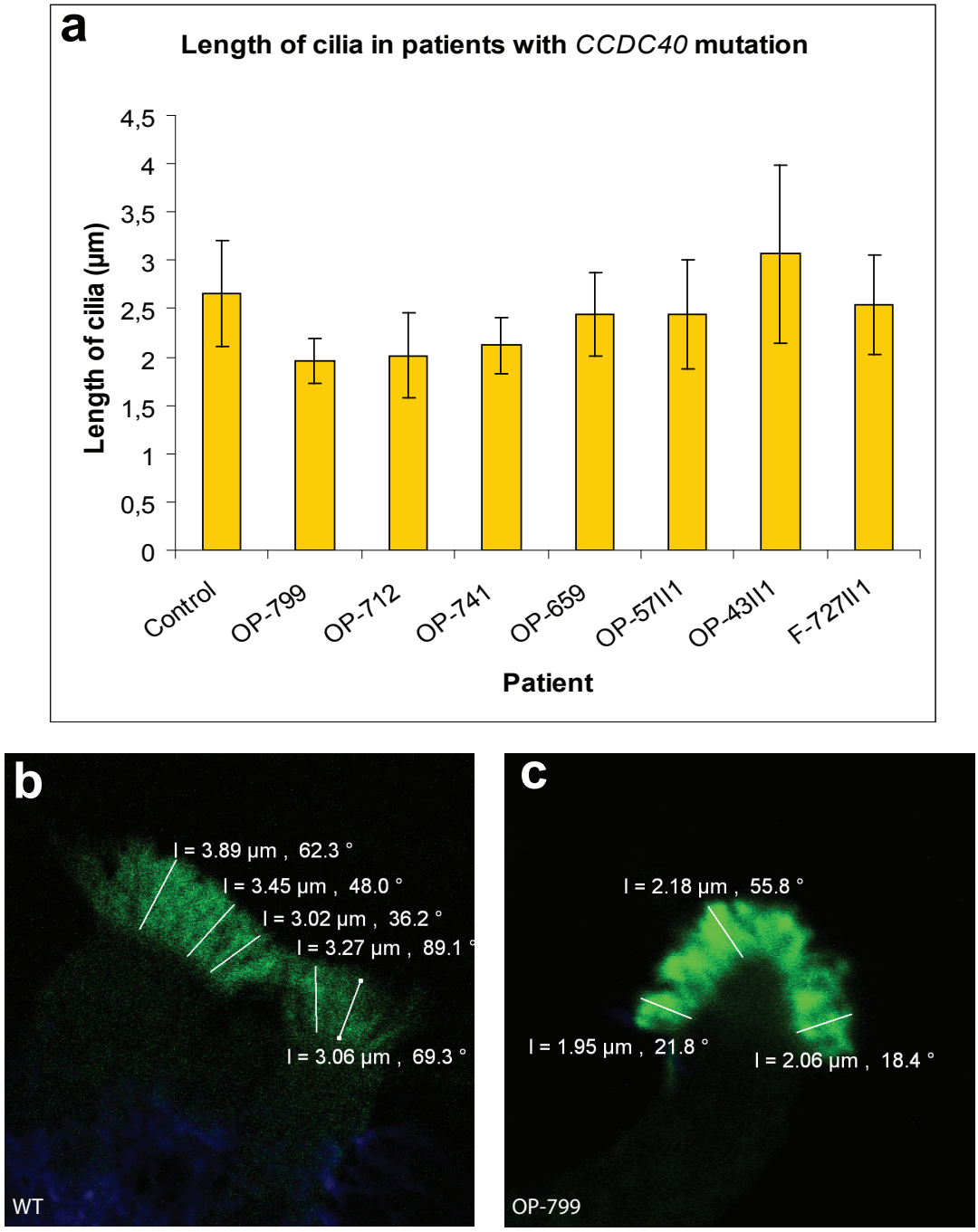

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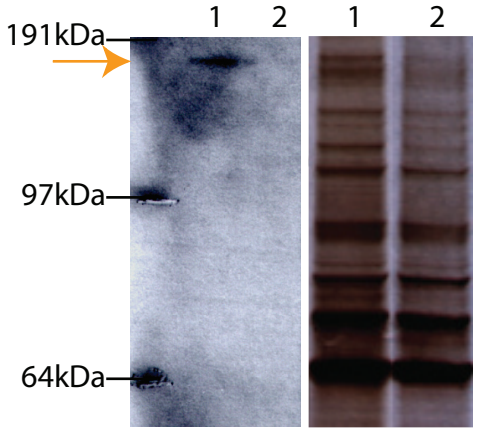

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Supplemental Table 1. Patients with *CCDC40* mutations

Patients	***Segregation***	***Electron microscopy***	***Ciliary DNALI1***	***Ciliary GAS11***	***Ciliary CCDC39***	***Ciliary DNAH5***	***High-speed videomicroscopy***	***Laterality phenotype***
F-727II1	P + M	n.a.	n.a.	negative	n.a.	normal	stiff with reduced amplitude	situs inversus
OP-120	n.a.	n.a.	negative	negative	negative	normal	stiff with reduced amplitude	situs solitus
OP-240II2	P + M	n.a.	n.a.	negative	n.a.	normal	stiff with reduced amplitude	situs inversus
OP-712II1	P + M	RS/CP- delocalization	n.a.	negative	n.a.	normal	n.a.	situs inversus
OP-712II2	P + M	RS/CP- delocalization	negative	n.a.	n.a.	n.a.	n.a.	situs inversus
OP-57II	P + M	RS/CP- delocalization	n.a.	negative	n.a.	normal	n.a.	situs inversus
OP-76II1	n.a.	n.a.	negative	negative	negative	normal	stiff with reduced amplitude	situs inversus
OP-87II2	n.d.	n.a.	n.a.	negative	n.a.	normal	stiff with reduced amplitude	situs solitus
OP-799	n.a.	RS/CP- delocalization	negative	negative	negative	normal	n.a.	situs inversus
OP-82II1	P + M	RS/CP- delocalization	n.a.	negative	n.a.	normal	stiff with reduced amplitude	situs inversus
OP-741	n.d.	RS/CP- delocalization	negative	negative	negative	normal	n.a.	situs solitus
OP-659	n.a.	RS/CP- delocalization	negative	negative	negative	normal	n.a.	situs inversus
OP-43II1	P + M	RS/CP- delocalization	n.a.	negative	n.a.	normal	n.a.	situs inversus
OP-43II2	P + M	RS/CP- delocalization	negative	negative	negative	normal	n.a.	situs solitus
OP-43II3	P + M	n..a	n.a.	negative	n.a.	normal	n.a.	situs solitus
OP-277II1	n.d.	n.a.	negative	negative	negative	normal	n.a.	situs inversus
F-677II1	n.d.	n.a.	n.a.	negative	n.a.	normal	stiff with reduced amplitude	situs inversus

Available clinical data do not indicate the presence of sensory hearing deficits, kidney cysts and/or hydrocephalus in affected patients harbouring *CCDC40* mutations. Data on sperm analyses are not available. Not a single loss-of-function mutation was reported in the latest 1,000 Genome Project data release (www.1000genome.org) for 60 sequenced individuals and the dbSNP (http://www.ncbi.nlm.nih.gov/snp/). CP = central pair; del – deletion; fs = frameshift; ins = insertion; IVS = inversion; M = maternal; n.a. = not available; n.d. = not determined; P = paternal; RS = radial spoke